

CTENIDIAL NUMBER IN RELATION TO SIZE IN CERTAIN
CHITONS, WITH A DISCUSSION OF ITS
PHYLETIC SIGNIFICANCE¹

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Before the discovery of the living monoplacophoran genus, *Neopilina*, in 1952, any summary diagnosis of the phylum Mollusca included such a phrase as "coelomate, totally lacking metamerie segmentation." This was completely acceptable despite the well-known replication of several sets of organs—including shell valves, pedal muscles and ctenidia—in the polyplacophoran Amphineura—the chitons. However, the detailed morphology of *Neopilina galathea*, so carefully described by Lemche and Wingstrand (1959b, see also Lemche, 1957), led these authors to propose that several features in both *Neopilina* and chitons were primitive metamerie characters providing undeniable evidence of the segmental origin of the phylum Mollusca. Certain workers with extensive knowledge of the functional morphology of molluscs have not been able to accept these characters as evidence of metamerie origins (Yonge, 1957; Morton and Yonge, 1964), regarding the multiplicity of certain organ systems (of cephalopods and chitons as well as of monoplacophorans) as being replication with functional, rather than segmentation with phyletic, significance. Other authors with extensive molluscan background (Fretter and Graham, 1962) have partially accepted the features as indications of metamerism and to some extent revived an older theory of molluscan origins. This older theory was developed as a result of studies of the genital and excretory systems of chitons and cephalopods (see Pelseneer, 1899, 1906; Naef, 1926), and it proposed a stem group of molluscs with short segmented bodies. For many years it seemed that this older theory had been completely dismissed as a result of the work on the functional morphology of primitive gastropods which culminated in the important survey by Yonge (1947), which established as the most likely ancestral mollusc a totally unsegmented animal with a posterior mantle-cavity and one pair of gills (ctenidia). The description of *Neopilina* has reopened discussion of possible metamerism in primitive molluscs, and, as outlined above, this has again involved consideration of the multiplied organ systems in chitons.

This paper presents data on the length-weight relationship and the ctenidial numbers found in *Chaetopleura apiculata* from Cape Cod and in *Lepidochitona cinereus* from the North Sea coast of Scotland, with some brief notes on conditions in certain other species of chitons. The data form part of other work by the present authors on the functional morphology of post-larval chitons (S. C. B.) and on

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variations in growth and fecundity in chitons (W. R. H.), but are presented here because of their significance in relation to the alleged metamerism of primitive molluscs.

MATERIALS AND METHODS

Two species of chitons were studied in detail. *Chactopleura apiculata* is the commonest chiton of the Atlantic seaboard of the northeastern United States, and was collected from the Northwest Gutter of Hadley Harbor and from Penzance, Buzzard's Bay, both near Woods Hole, Massachusetts. *Lepidochitona cinereus* is one of the four commoner intertidal chitons of British waters, and was collected from rock pools at Leckmoram Ness on the Firth of Forth, near North Berwick, Scotland. Both chitons were collected from between low-water of neap tides and low-water of springs. The species and collection localities of the other chitons not studied in detail are given in the text where they are mentioned. Initial ctenidial counts were made on living chitons and on material fixed and preserved by various standard procedures. The principal material of both *Chactopleura* and *Lepidochitona* used to establish the length-weight relationship and to count gills was prepared by a method which produces maximum flexibility of the ctenidia. A similar method has been employed extensively by one of the present authors (W. R. H.) in work on slugs and other pulmonate gastropods, and an account of this use was given by Owen and Steedman (1958; see also Rosewater, 1963, for further references). In the case of the chitons, they were placed in fingerbowls of clean sea water and propylene phenoxetol⁴ gently added to form globules on the bottom of each dish (not touching the chitons). The amount of propylene phenoxetol added was about 0.1–0.2% of the volume of sea water. They were then left undisturbed, in dim light, for about eight hours. Fixation of these partially-narcotized chitons was then carried out in 12% formalin in sea water, each being held flattened against a glass surface when first immersed in the fixative. They required to be held for only about 10–15 seconds to prevent curling. Fixation was continued for four hours, and the chitons then washed for four hours in running water. Finally they were transferred to the storage fluid: an aqueous solution of 1% propylene phenoxetol, and 1% ethyl alcohol in 10% glycerol. Animals prepared in this way have life-like tissues, soft, flexible and excellent for dissection. For the benefit of anyone applying this method to other material, it should be emphasized that propylene phenoxetol has been used only (1) as a relaxing agent, and (2) as a preserving fluid (actually as a bacteriostatic agent). It should be used in the latter capacity only after adequate fixation (since it does not prevent tissue autolysis, etc.). In this work, chiton length was taken as the maximum distance along the midline from the anterior to the posterior "girdle" edges of flattened specimens. Length measurements were made to the nearest 0.1 mm. with an engineer's dial caliper. Weights were "wet tissue weights" determined to the nearest milligram after removing excess external water with filter paper, on a precision micro-torsion balance (V.D.F., Holland) with a scale of 1 gm. in milligrams. Ctenidia were counted under a dissecting microscope at about 20×, using high-intensity incident illumination, by leafing over with a fine dissecting needle. All counts were checked, "asymmetrical" chitons re-checked and significant counts double-checked by both

⁴ U. S. distributor: Goldschmidt Chemical Corp., 153 Waverly Place, New York 14, New York.

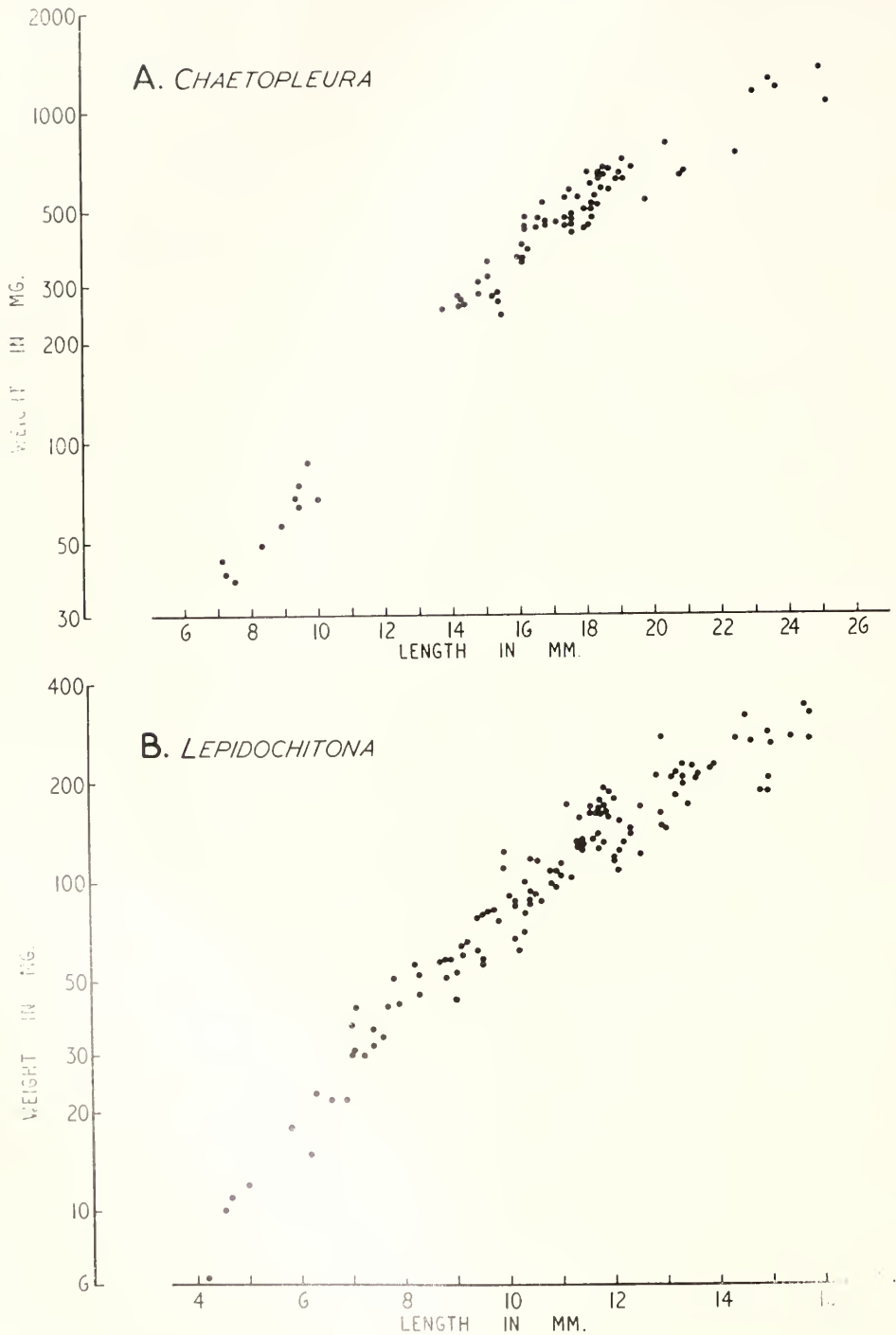


FIGURE 1. "Wet tissue weight" in relation to body length in chitons (weights in milligrams on the ordinate are plotted logarithmically). 1A, for *Chaetopleura apiculata* from Woods Hole, Mass.; 1B, for *Lepidochitona cinereus* from the Firth of Forth, Scotland.

authors. Those chitons with obvious pallial or pedal damage (or signs of regeneration), and those few with an obvious gap in the gill series, were rejected and not included in the ctenidial counts. "New" ctenidia in the process of formation presented difficulties. Tiny dome-shaped buds lacking any obvious structure *were not* counted, but even the smallest gill rudiments showing differentiation of an axis with two or more ctenidial leaflets *were* counted.

RESULTS

The "best" measures of size and growth in chitons utilize volume or mass. Linear measurements of the whole animals involve several sources of error, including variations in flattening and in the degree of expansion of the mantle edge. Attempts have been made to avoid these errors by using measurement of individual plates (see Arey and Crozier, 1919), or along the plate series. The method of preparation outlined above results in flat, little-contracted chitons whose lengths can be readily and reproducibly determined. Figures 1A and 1B show the relation of length to wet tissue weight for *Chaetopleura apiculata* and for *Lepidochitona cinerea*. The relation of weight to length calculated for *Chaetopleura* is $W = 0.0881 L^3$, and for *Lepidochitona* is $W = 0.0833 L^3$ (with W in mg., and L in mm.). That is to say, the shapes and relative densities of *Chaetopleura* and of *Lepidochitona* (and indeed of most other chitons) are closely similar to each other.

These weights are used in Figures 2A and 2B, in which they are plotted against the ctenidial numbers actually counted in each specimen. Bolder points indicate chitons which are symmetrical, *i.e.*, have the same number of gills on the left as on the right side. Horizontal lines indicate asymmetric chitons and link the appropriate gill numbers. In both species there is clearly a correlation between gill number and size. In the sample of *Chaetopleura apiculata* (Fig. 2A) weighed and counted, 58 out of 76 are symmetrical. In some of these numerically symmetrical specimens, however, there is marked asymmetry in the size of the anteriormost gill or gills. In adult specimens of *Chaetopleura*, as in all chitons, these are the most recently formed. Although there is clearly a correlation of increase in ctenidial number with increase in weight (Fig. 2A), there is no direct evidence available to link weight (or any other size measurement) with age in *Chaetopleura apiculata*. Circumstantial evidence suggests that in *Lepidochitona cinereus*, and in other relatively small chitons like *Chaetopleura*, the length of life is between three and six years (see, however, Comfort, 1957). From the sample of *Chaetopleura apiculata* used in Figure 1A and 2A, and from other samples measured during studies on fecundity, the only clearly marked "generation" is made up of specimens less than 12 mm. long, *i.e.*, under 100 mg. weight, in late summer samples. Later generations (200 mg. to 1.5 g. wet weight) are inseparable on presently available data. It should be noted that this group of small chitons (almost certainly about 12 months old in late summer) already have from 17 to 20 gills on either side. There is a gap in our knowledge of *Chaetopleura* between the immediate post-larval stages and these young "year-old" adults. Recent work (S. C. B., unpublished) has shown that no ctenidia are present in the pallial grooves of the 30-day post-larva (which has the full eight shell plates). However, from the sixth day to the 33rd day post-larva, water currents created by ciliated epithelia in the pallial grooves are functionally analogous to those found in the adult, in which they are produced by

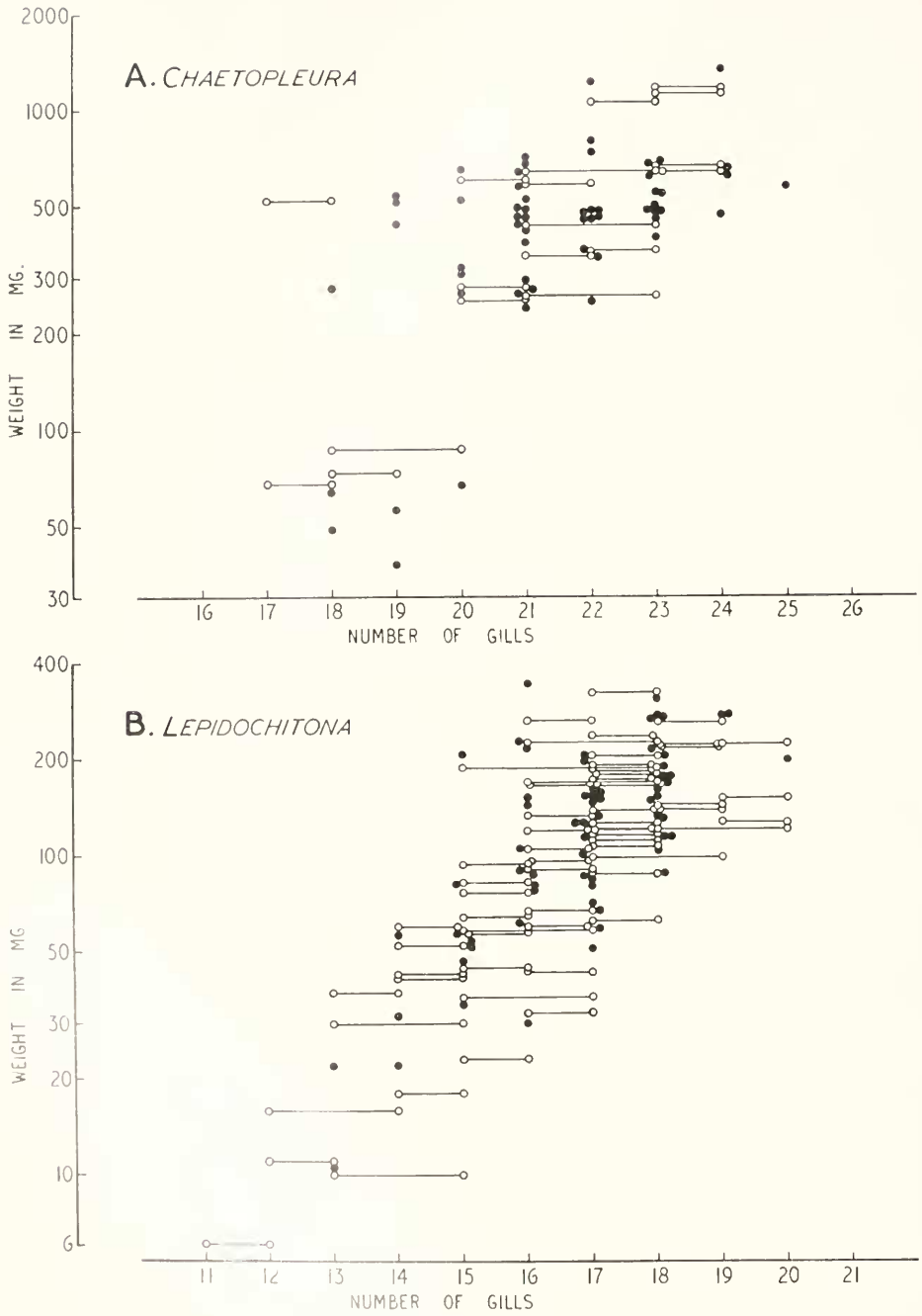


FIGURE 2.

the lateral cilia on the faces of the ctenidial leaflets. It is hoped to investigate the later post-larval development, particularly with regard to ctenidial morphogenesis and ciliation. Meanwhile, in functional terms, it can be assumed that the surface-volume ratio in the 30-day chiton (measuring 0.55 mm. long, and assessed at 14.4 μ g. live weight) is such that the animal has no respiratory "need" to develop the elaborate increase in surface area provided by ctenidia. The last large ctenidium in young adult specimens of *Chaetopleura* is the third from the posterior end in each ctenidial row (*i.e.*, immediately behind the renal opening, and the second gill behind the genital opening). This particular gill is almost certainly the first formed and a definite number (two) of ctenidia are added behind, while a less definite number (from 14 to 22 in our samples) are added anteriorly. Asymmetry in gill numbers involves only the anteriorly-added ctenidia, *i.e.*, no trace of numerical asymmetry involving the posterior gills was detected—either in *Chaetopleura apiculata* or *Lepidochitona cinereus*.

A further pallial variation was noted in *Chaetopleura*. Although critical measurements were not made, a subjective but positive impression was gained that within the populations of *Chaetopleura* studied, two distinct forms occur—differing in the extent to which the pallial grooves are occupied by ctenidia. This was irrespective of total ctenidial number and size. In one type, the bases of the gills extend forward to the level of the head fold. This would conform to the accepted diagnosis of the genus *Chaetopleura*. In the other form, ctenidial bases extend forward for only two-thirds of the length of the pallial grooves (*i.e.*, to the level of the anterior quarter of the foot). Thus in this form, the anterior third of the pallial groove on each side is a naked gutter, and the whole pallial arrangement approximates that pertaining in chitons like *Lepidopleurus*. In the sample on which Figure 2A is based, 18 out of 76 specimens of *Chaetopleura* were asymmetric in ctenidial numbers. An earlier count of *Chaetopleura* from the same locality, which cannot be shown in the above figure since weights were not determined, gave three asymmetric out of 32. This gives a grand total for *Chaetopleura* of 21 asymmetric out of 108, or 19.5%.

Lepidochitona cinereus was chosen among British chitons, both because a preliminary survey showed a high incidence of asymmetry in the species, and because a population was already being sampled for gonadal studies. Figure 2B shows that only 65 out of a sample of 126 chitons were symmetrical, and that the asymmetries in one case involved a difference of three gills (15:18), in nine cases involved a difference of two gills, and in 51 cases a difference of one gill. Actual asymmetries are commonly more extensive than the figures for *Lepidochitona cinereus* indicate. Thus, often an 18:18, or 17:17, or 16:16 count includes one relatively large anterior (last-formed) gill on one side, and a minute (though differentiated) anterior gill on the other. In *Lepidochitona* as in *Chaetopleura*, there is clearly a correlation between adult size, expressed as wet tissue weight, and

FIGURE 2. Number of ctenidia on each side of the body in relation to "wet tissue weight" in chitons (weights in milligrams on the ordinate are plotted logarithmically); closed circles (●) indicate chitons which are symmetrical (*i.e.*, which have the same number of ctenidia on left and right sides); horizontal lines indicate asymmetrical chitons and link open circles (○) at the appropriate gill numbers. 2A, for *Chaetopleura apiculata* from Woods Hole, Mass.; 2B, for *Lepidochitona cinereus* from the Firth of Forth, Scotland.

ctenidial number. For *L. cinereus* in this particular population, there is rather better circumstantial evidence relating size to age. When the weight distribution of Figure 1B is plotted on probability paper (as used by Harding, 1949, and applied by Hunter, 1961, to snail populations) it shows a polymodal distribution with four significant peaks. However, length distributions for several other samples from this population were prepared in connection with studies on fecundity and, when these are plotted, show a clear trimodality which can be attributed to a 1+ (*i.e.*, more than one year old, *e.g.*, in a September sample such as the one used for weights and ctenidial counts—about 14–15 months old), a 2+ and a 3+ age group. It is notable that the youngest (1+) specimens of *Lepidochitona* already have from 11 to 15 gills on each side. No immediately post-larval material of *Lepidochitona* was available. In summary, numerical asymmetry in *Lepidochitona* involved 61 out of a sample of 126 individuals or 48.4% of the population, and qualitative asymmetry involved a still greater proportion of the population.

Certain general observations can be made. Except for a few very small specimens of each species, most of the *Chaetopleura* and *Lepidochitona* examined were adult. Growth continues in adult chitons, and apparently the development of

TABLE I
Extent of ctenidial asymmetry in two species of chitons

	Nos. of individuals							Totals
	With extra left gills			Symmetric	With extra right gills			
	3	2	1		1	2	3	
<i>Chaetopleura apiculata</i>	0	2	8	87	9	2	0	108
<i>Lepidochitona cinereus</i>	0	5	24	65	27	4	1	126

additional ctenidia anteriorly also continues. There is a real correlation between gill number and adult size. However, in these samples at least, there is no indication of any increase in asymmetry with increasing size. The relation of left to right sides in both species is summarized in Table I.

In the course of gill-counting in *Chaetopleura* and in *Lepidochitona*, the few specimens which showed damage to a ctenidial row, or repair thereof, were rejected. In the 234 specimens of the main series, three cases were encountered in which ctenidial axes were split. In one specimen of *Chaetopleura*, the last (posterior) gill on the left side was biaxial, and in another, the third and fourth gills from the posterior end of the right row were both triaxial. In one specimen of *Lepidochitona* the last gill on the right side was triaxial.

Certain other chitons were examined in less detail. These included small samples of four other species of chitons from Scottish waters, which were examined before the main series of *Lepidochitona cinereus* was prepared. Eleven specimens of *Lepidopleurus cancellatus*, dredged from 18 m. off Dun Chonnuill in the Garvelloch Islands (see Hunter and Muir, 1954), had lengths ranging from 3.3 to 7.6 mm., had gill numbers from 8 to 11 per side, and included three asymmetric

specimens (11L:10R, 11L:10R, and 9L:10R). A fresh sample of the commoner Clyde species of this genus (*L. asellus*) was not available but six specimens in a museum collection proved to be all symmetrical with from 10 to 13 gills on each side. A sample of nine specimens of *Tonicella marmorca* from near Shandon, on the eastern shore of Gareloch, ranged in size from 12.5 to 23.7 mm., had gill numbers from 17 to 28 per side, and included one asymmetric individual (23L:24R). Samples of *Lepidochitona cinereus* from this last locality, and from two localities in the Firth of Forth showed that nearly half of each population was asymmetric in gill numbers. Since *L. cinereus* is also the most abundant intertidal Scottish chiton, it was decided to use this species along with *Chactopleura apiculata* in the main series. Lastly, 15 specimens of *Acanthochitona crinitus* (formerly known as *Acanthochitona fascicularis*), collected from near low water mark on a boulder beach just south of Port Appin, Argyllshire, ranged in length from 6.1 to 13.0 mm., had gill numbers from 14 to 17 per side, and included two asymmetric individuals (both 15L:16R). Thus, of five species of Scottish chitons examined, four proved asymmetric in ctenidial numbers. It is worth noting that these five species include representatives of both the major orders of living Polyplacophora. Three genera: *Lepidochitona* and *Tonicella* (both in family Lepidochitonidae), and *Acanthochitona* (family Cryptoplacidae) are placed in the Order Chitonida, the group containing the more typical "modern" chitons usually living on rocky surfaces in the intertidal zone. The genus *Lepidopleurus* (family Lepidopleuridae) is placed in the Order Lepidopleurida, which includes some of the most ancient of polyplacophoran genera (e.g., *Hcluinthochiton*, from Lower Ordovician) as well as four surviving genera which live offshore and in deeper waters (extending to beyond 6000 m.).

Subsequently, commercially obtained samples totalling 65 specimens of a moderately large chiton, *Katherina tunicata* from the Pacific Coast, probably collected near Gladstone, Oregon, were examined. Of these, 24 specimens proved to have ctenidial or pallial damage, and weights and gill counts were determined on the remaining 41. Wet weights ranged from 13.8 to 55.8 g., and gill numbers from 46 to 61 per side. Only 22 out of 41 specimens of *Katherina* were symmetrical, and the asymmetries (46.3% of the population) in two cases involved a difference of three gills, in five cases a difference of two gills, and in 12 cases a difference of one gill.

Few previous authors have studied ctenidial numbers in chitons. In a footnote, Pelseener (1897) refers to his finding single specimens of six species of Atlantic chitons, each with an asymmetry of one gill. His species were *Acanthochitona discrepans*, *A. zelandicus*, *Lepidopleurus cajetanus*, *L. articus*, "*Borochiton marmorcus*" (= *Tonicella marmorca*, above), and *Ploxiphora coelata*. Perhaps the only previous detailed examination of a population was by Snyder and Crozier (1922) on *Chiton tuberculatus* at Bermuda. These authors examined 100 specimens, and in individuals ranging in size from one to ten cm. long, found ctenidial numbers from 32 to 54 on each side with 69% of the sample asymmetrical.

Thus, in four species of chitons studied in detail, the percentages of asymmetrical specimens found have been 19.5% (*Chactopleura apiculata*), 46.3% (*Katherina tunicata*), 48.4% (*Lepidochitona cinereus*), and 69% (*Chiton tuberculatus*). In addition, asymmetries of ctenidial numbers occur in at least eight other species.

DISCUSSION

As noted in the introduction, the discovery of *Neopilina* has reopened discussion of possible metamerism in ancestral molluscs. Chitons show replication in several organ systems—including shell valves, pedal musculature, nervous system, circulatory system, and ctenidia. As a result of their anatomical studies of *Neopilina*, Lemche and Wingstrand (1959b; see also Lemche, 1957, 1959b) proposed that such features of chitons, as well as similar structures in *Neopilina*, were primitive metameric characters. Lemche (1959a, 1959c) has gone so far as to detail homologies between a primitive arthropod triramous appendage and the gill in *Neopilina*, and between this latter and the ctenidia in the rest of the Mollusca (Lemche, 1959b).

Of course, the hypothesis that the molluscan ancestor was a short segmented animal has been advanced before (see, *e.g.*, Pelseneer, 1899, 1906; Naef, 1926). These older protagonists of a segmented ancestor drew their evidence largely from morphological studies of the coelomic derivatives, notably reno-pericardial and genital ducts, in chitons and cephalopods. The significance of *Neopilina* in relation to such theories was stressed by Lemche and Wingstrand (1959a, 1959b). They suggested that the ancestral mollusc must have shown relatively complete metamerism, that this is present to a somewhat reduced extent in *Neopilina*, that this is still further reduced in chitons, and that this metamerism degenerates so completely as to be undetectable in the rest of the molluscs.

For about 30 years the original metameric hypothesis, set up by Naef and Pelseneer among others, was abandoned. This resulted from the extensive and convincing work of the molluscan comparative morphologists, such as Yonge, Graham, and Fretter, whose studies of ciliary mechanisms, ctenidial blood-vessels, reno-pericardial and genital ducts in the more primitive gastropods (as summarized in Yonge, 1947) indicated a very different pattern of ancestral mollusc. Although primitively bilaterally symmetrical, this animal was totally unsegmented and possessed a posterior mantle-cavity enclosing a pallial complex of paired structures which included two ctenidia. The setting up of such a hypothetical ancestor had, of course, required consideration of those cases of molluscs in which there are more than two ctenidia, *i.e.*, the primitive cephalopod *Nautilus* (see Yonge, 1947) and all the chitons (Yonge, 1939). Such consideration led to the conclusion that these cases represent *secondary* replications of structures of the ancestral mollusc. The publication of the beautifully detailed anatomical account of *Neopilina galathea* (Lemche and Wingstrand, 1959b), has made review of concepts of molluscan evolution inevitable. Few students of the Mollusca would accept the more extreme homologies proposed by Lemche (1959a, 1959b, 1959c), which equate structures in the more primitive molluscs with corresponding annelid parts. Fretter and Graham (1962), in their excellent and detailed synthesis of existing knowledge of functional morphology in the prosobranch gastropods, have accepted the concept that *Neopilina* shows metamerism and have linked this to the older theory of a segmental ancestor. They also support the concept that the mantle-cavity was most primitively a groove bounded by the mantle edge and surrounding the head-foot, rather than a posterior cavity. Yonge (see Morton and Yonge, 1964) has denied that metamerism occurs in *Neopilina* and, although allowing that the ancestral mantle-cavity might have been a pallial groove, adheres essentially to the concept of the ancestral mollusc

outlined above. Obviously, aspects of these theories are mutually exclusive. The pattern of molluscan evolution, involving successive reductions of metameric structures, proposed by Lemche and Wingstrand (1959a, 1959b) requires as a premise that the multiplied organ-systems of chitons represent a simplification of more extensive metamerism.

Therefore, the question must be asked: do chitons show any vestiges of true metamerism? A seemingly negative answer is provided by existing evidence on the development of shell plates, on variation in auriculoventricular connections in the Class, and by the data on ctenidial numbers presented in this paper.

As regards shell plates, their development in several chitons is similar. The elongate trochophore develops a mantle rudiment, which about the time of settlement secretes six shell plates. After an interval, a larger plate is added *anterior* to the series. Still later, a small last plate is added at the *posterior* end of the series. At no stage in the development is there a "budding zone" for shell plates. This sequence of events, known for several chitons, has recently been confirmed for *Chaetopleura apiculata* by one of the present authors (S. C. B.).

As a Class, the chitons show significant variation in the afferent chambers of the heart. These variations were surveyed by Pelseneer (1897, 1906). The pericardium is always dorsal and posterior, and contains an elongate ventricle in the midline which receives blood from two symmetrical elongate auricles. In a minority of chiton genera, Pelseneer found that there are single auriculoventricular (A-V) openings on either side; in the majority of genera there are two A-V openings on either side; while in *Chiton squamosus* there are three pairs and in *C. goodalli* four. Pelseneer also remarks that numerical asymmetries of the A-V openings can occur, although they are apparently extremely rare. It would be difficult to relate the paired auriculoventricular openings to any external "segmentation" such as shell valves. Similarly, although the renal organs of chitons are lobulated, relating particular lobes to external "segments" or to the renal lobes and discrete "nephridiopores" of *Necopilina* would be equally difficult.

Turning to the ctenidia of chitons, their numbers, though approximately fixed for each species, show variations which are germane to the question of metamerism. First, gills are added anteriorly and somewhat irregularly as growth proceeds. Second, there can be marked asymmetry between left and right ctenidial rows in individual specimens (from 19.5% to 69% in four species studied in detail). Third, actual asymmetry of development can be more marked than mere enumeration of ctenidia would indicate, since the degree of differentiation of the anterior-most gills often varies. Fourth, the "definitive" ctenidial number in different species of chitons ranges from 8 to 160. (No one has ever suggested a primitive mollusc with 80 segments.) Finally, individual ctenidia cannot be related to any other replicated structure, still less allocated to specific segments.

The broad phyletic significance of the above can now be summarized. If no true metamerism occurs in primitive molluscs, the closest connections and possible origins of the phylum lie in the turbellarian-rhynchocoel phyla (as is concluded by Yonge, 1947; Morton and Yonge, 1964). If metamerism was a feature of the primitive mollusc, connections should be sought with the annelid-arthropod phyla (as urged by Lemche and Wingstrand, and partially accepted by Fretter and Graham, 1962). No one has attempted to establish a connection between

molluscs and either of the other metazoan groups showing segmentation: the Cestoda or the Chordata. At this point, it is appropriate to try to define metameric segmentation as it is found in the Annelida and Arthropoda. Hyman (1951, pp. 28-29) offers several cogent phrases in defining segmentation as "a serial succession of sections, each of which contains identical or similar representatives of all the organ systems of the body" in which "all parts . . . are serially repeated at regular intervals" and "the segments form in an anteroposterior sequence so that the first segment is the oldest, the last the youngest." Thus, the essence of metamerism is the serial succession of segments, each containing unit-subdivisions of the several organ systems.

The most detailed homologies between chitons and *Ncopilina* proposed by Lemche and Wingstrand (1959) involved the alleged metamerism of the gills and circulatory organs of both (although clearer "segmentation" in *Ncopilina* is found in the coelomic cavities and their genito-renal derivatives). A major implication of the results presented above is that "segmentation" in chitons is more apparent than real. It is difficult to imagine morphogenesis of any metameric sort which would allow the addition *anteriorly* of the penultimate shell valve to the six already developed, and this followed finally by the laying down of the last (eighth) valve *posteriorly*. Similarly it is difficult to invoke metamerism with regard to the anterior addition, irregularly and independently on either side, of ctenidia as growth proceeds. Finally, it is difficult to characterize as a metamERICALLY segmented animal: a chiton with 8 shell plates, with two, three or four auriculoventricular openings, with 21 ctenidia on one side and 23 on the other (or 15 and 18), and with a ladder-like nervous system of irregular transverse connectives—all these arranged apparently independently of each other. There is little of the serial succession of segments, each containing unit-subdivisions of the several organ systems, about such replications as are found in chitons. Thus, it seems most probable that the multiplied structures of chitons reflect functional replication rather than ancestral segmentation.

Even if we can thus dismiss true metamerism as regards chitons, the question of "segmentation," both in *Ncopilina* and in a hypothetical molluscan ancestor, remains. Similar arguments to those presented above can be used to criticize the concept of metamerism applied to the described structures of *Ncopilina*. *Ncopilina* has five pairs of gills, two pairs of auricles, six pairs of nephridia, one or possibly two pairs of gonads, 8 pairs of pedal retractor muscles, 10 sets of lateropedal nerve connections, and a single shell with a coiled protoconch. As Yonge has pointed out (Yonge, 1957, 1960; Morton and Yonge, 1964), final assessment of the phyletic position of *Ncopilina* must await embryological studies—particularly on the development of the allegedly metameric structures, and functional studies on the organization of the gills in living *Ncopilina*, which could establish whether or not these structures are homologous with the ctenidia of the other molluscs.

The question of the ancestral stock can be re-examined. According to the classical picture, derived from studies on the functional morphology of the most primitive living gastropods (Yonge, 1947), the ancestral mollusc had a posterior mantle-cavity containing one pair of ctenidia connected to one pair of auricles. However, even before the discovery of *Ncopilina*, there were several difficulties in relating this model to conditions in the primitive cephalopod *Nautilus*—with two

pairs of ctenidia and four auricles, and in the chitons—with many ctenidia and two elongate auricles (with two, four, or six auriculoventricular openings). There is a striking resemblance in hearts (each with four auricles and a "single" ventricle) between *Ncopilina* and *Nautilus*. There is a further, though less obvious, resemblance of both to conditions as they exist in the majority of chitons—with four auriculoventricular connections. Perhaps surprisingly, the arrangement of heart chambers and of their interconnections seems to form a relatively conservative feature in the evolution of other molluscan groups. For example, within the Order Neritacea, evolution of fresh-water and terrestrial forms has occurred completely independently of—though occasionally parallel to—the rest of the gastropods (Morton and Yonge, 1964; Hunter, 1964). The course of this evolution has involved the usual gastropod reduction of symmetrically paired structures. However, comparative studies on certain Neritacea by one of the present authors (W. R. H., unpublished) have shown that even in many forms where this reduction is complete for almost all of the other structures, a small right auricle remains, in addition to the functionally important left one.

In a footnote in the classic paper of Yonge (1947), Dr. C. F. A. Pantin inquired if it was necessary to postulate the possession of only a single pair of ctenidia in the primitive mollusc. In view of the heart morphology of *Ncopilina*, *Nautilus* and chitons discussed above, it would seem as justifiable to set up a model ancestral mollusc with four ctenidia and therefore four auricles. Subjectively, a four-fold basic organization would seem a more reasonable starting point for two sorts of morphogenesis: involving either reduction to one pair or replication to many. Both a line of organisms with one gill on either side, and a line with many could thus evolve from an ancestral stock with two gills on either side.

While it is realized that the setting up of yet another hypothesis, with four as the ctenidial number in the stem-mollusc, is but tenuously based on the observed phenomena of replication of parts in chitons, it is emphasized that the conclusion that chitons are *not* metameric is much more firmly established.

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SUMMARY

1. The discovery and description of *Ncopilina* has reopened discussion of possible metameric segmentation in the primitive molluscs. This has involved reconsideration of the multiplied organ systems in chitons, which some authorities regard as metameric in origin. The length-weight relationship was determined and ctenidial numbers counted for populations of two species of chitons: *Chactopleura apiculata* from Cape Cod, and *Lepidochitona cinereus* from the North Sea coast of Scotland. Less detailed observations were made on other species of chitons and on other organ systems. Growth continues in adult chitons, together with the addition of ctenidia anteriorly, so that there is a correlation between gill-number and adult size. *Chactopleura apiculata* adults, weighing from 38 to 1324 mg., had

gill-numbers ranging from 17 to 25 per side. *Lepidochitona cinereus* adults, weighing from 6 to 340 mg., had gill-numbers ranging from 11 to 20 per side. Asymmetry in ctenidial numbers between the left and right sides of single specimens occurred in 19.5% of *Chactopleura apiculata*, and in 48.4% of *Lepidochitona cinereus*. Qualitative asymmetry was even more extensive in the populations studied. Since such marked ctenidial asymmetries as reported above occur, and since individual gills are added independently on either side, the ctenidia of chitons *cannot* be paired structures. The organization of certain other replicated structures (including shell-plates, auriculoventricular connections, and renal lobes) is considered more briefly, drawing on earlier pertinent work, and leads to the obvious result that individual ctenidia cannot be related to any other replicated structure—still less allocated to specific “segments.”

2. These findings are discussed in relation to the alleged metamerism of chitons, and it is also concluded that such replicated organs *cannot* represent a simplification of a more extensive metamerism. Further, it is shown that it is possible to criticize the concept of metamerism as applied to the described structures of *Neopilina*. The phyletic significance of this is explored, and it is concluded that evidences for a connection between the primitive molluscs and the turbellarian-rhynchocoela phyla are better than are those for metamerism and an annelid-arthropod connection. A model ancestral mollusc with a *four-fold* basic organization (e.g., four ctenidia, four auricles, four renal organs, etc.) is proposed. It is stressed that, while *any* model of an ancestral mollusc is highly speculative, the evidences against metamerism in chitons (and probably in all primitive molluscs) are overwhelmingly strong.

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